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(54) **SOLUBILIZATION OF HYDROPHOBIC MATERIALS USING LYSOPHOSPHOLIPID.**

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Description

The present invention is directed to the field of solubilization of hydrophobic materials. More particularly, the invention describes the use of certain lysophospholipids as solubilizers and non-toxic delivery vehicles.

The solubilization of hydrophobic materials, particularly bioactive materials, is typically achieved by the use of surfactants such as sodium deoxycholate or propylene glycol. Such surfactants, due to their detergent properties, are biologically incompatible and toxic due to their lytic effects on cells. It would, therefore, be desirable to employ a naturally occurring substance without toxic properties as a surfactant for pharmaceutical use.

Lysophospholipids have heretofore been improbable candidates for pharmaceutical excipients due to their lytic effects on cells. We describe a method and compositions for a lysophosphatide, specifically lysophosphatidylethanolamine, alone and in combination with an unsaturated phospholipid for solubilizing hydrophobic materials.

Peterson et al., Arch Biochem Biophys, 179, 218-228 (1977), observed lysophosphatidylethanolamine's (LPE) properties, as an ATPase inhibitor in biomembranes; these effects probably due to LPE intrusion into the membrane around the enzyme resulting in a less fluid lipid environment. Lysophospholipid suspensions were mixed with fractions of sarcoplasmic reticulum and changes in ATPase activity were recorded using a spectrophotometric assay. LPE, however, unlike lysophosphatidylcholine, never solubilized biomembrane at any concentration.

The present invention exploits the pH and temperature dependent phase transitions of lysophosphatidylethanolamines to result in micellar solubilization of hydrophobic materials and delivery of a non-toxic product.

The present invention describes solubilization of hydrophobic substances using LPE alone or LPE in combination with an unsaturated phospholipid.

The LPE's of the present invention have the formula

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and between 1 and 6 double bonds, preferably 13-19 carbon atoms, and more preferably 15-17 carbon atoms and 1-3 double bonds. A preferred LPE is that wherein RCO_2 is 1-oleoyl, as in formula I above, where the oleoyl group is bound to the 1 carbon atom.

A phospholipid is a glycerol molecule having one hydroxyl esterified to phosphoric acid which is further esterified to an alcohol component; and the other glycerol hydroxyls are esterfied to carboxylate fatty acid chains. The unsaturated phospholipids used in the present invention contain at least one fatty acid chain of between 12 and 22 carbon atoms and 1 to 6 double bonds, preferably 16 to 20 carbon atoms and 1 to 3 double bonds. The second fatty acid chain contains 12 to 22 carbon atoms and 0 to 6 double bonds, preferably 14 to 18 carbon atoms and 0 to 2 double bonds, more preferably 0 or 1 double bonds. Suitable phospholipids include, but are not limited to, derivatives of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol or phosphatidylcholine. Preferred unsaturated phospholipids include egg phosphatidylcholine, soy phosphatidylcholine or dioleoylphosphatidylcholine.

The lysophospholipid, LPE, alone or in combination with a phospholipid, is mixed with the hydrophobic material in an aqueous medium at a temperature of between 1°C and 90°C, and at pH between 8.5 and 14.0, preferably 25°C and pH 8.5. Alternatively, a more highly unsaturated LPE may be used; in this case, the hydrophobic material may be mixed with the lipid and aqueous medium at a temperature of between 0°C and 90°C, preferably 25°C and at pH 7.0. The temperature of the suspension is then reduced to below 0°C. The hydrophobic material can comprise bioactive agents including, but not limited to, drugs, hormones, proteins, dyes, vitamins or imaging agents. The aqueous medium can comprise a buffer system such as borate or N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid (HEPES). The resulting suspension may be passed under pressure through a filter system such as stacked polycarbonate filters and may be sonicated to further disperse the hydrophobic material.

OBJECT OF THE INVENTION

An object of the invention is a composition comprising an aqueous micellar solution at between pH 8.2 to 14.0 of a hydrophobic material and a lysophospholipid of the formula :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 1 double bond. Preferably, this composition comprises an aqueous solution at a pH of 8.2 to 8.5.

Another composition may comprise an aqueous micellar solution at between pH 6.0 to 8.0 of a hydrophobic material and a lysophospholipid of the formula :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 2 to 6 double bonds, and wherein the temperature of the composition is between -20 °C and 0 °C. Preferably this last composition may comprise an aqueous solution at a pH of 7.0.

Another object of the present invention is a method of preparation of a composition which comprises solubilizing a hydrophobic material with the steps of :

(a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of a lysophosphatidylethanol-amine of the formula :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

and hydrophobic material are dissolved to obtain a film ; and

(b) hydrating the film with an aqueous medium at pH of between 8.2 and 14.0 ; and

(c) admixing the aqueous medium and the hydrated film comprising the hydrophobic material and the lysophosphatidylethanolamine,

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 1 double bond.

Another method of preparation of a composition, according to the invention, comprises solubilizing a hydrophobic material with the steps of :

(a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of lysophosphatidylethanolamine of the formula :

$H_2C(OH)CH\text{-}O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

is dissolved to obtain a film ;

(b) hydrating the film with an aqueous medium at pH of between 6.0 and 8.0 ; and

(c) admixing the hydrophobic material with the hydrated film of step (b) ; and

(d) cooling the dispersed lipid in aqueous medium to a temperature below 0 °C ;

wherein R is a hydrocarbon chain having between. 11 and 21 carbon atoms and 2 to 6 double bonds.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 are 81MHz ^{31}p-NMR spectra of aqueous dispersions of 1-oleoyl lysophosphatidylethanolamine (LOPE, or sn-1-18 : 1_{cis}-PE) at pH 7,0 between - 20 °C and 90 °C.

FIGURE 2 are ^{31}P-NMR spectra showing the effect of pH variation on the polymorphic phase behavior of LOPE.

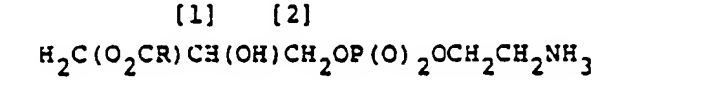
FIGURE 3 are ^{31}P-NMR spectra showing the effect of temperature variation on the polymorphic phase behavior of (A),sn-1-18:2$_{cis}$-PE and (B) sn-1-18:3$_{cis}$-PE.

FIGURE 4 is a graph depicting the hemolytic properties of various phospholipids.

FIGURE 5 is an expanded scale of hemolytic properties of various phospholipids.

DETAILED DESCRIPTION OF THE INVENTION

1-oleoyl lysophosphatidylethanolamine may be expressed as



wherein R is the oleoyl group attached to the carbon in the 1-position, as labeled [1], thus 1-oleoyl. The (OH) group is located on the [2] carbon. This lipid may be further expressed as sn-1-18:1$_{cis}$-PE, denoting the 18 carbon composition of the oleoyl group, followed by a number denoting the number of double bonds, in ths case 1 double bond in the cis configuration. As a further illustration of the nomenclature, for example, more highly unsaturated LPE's, wherein R is in the 1-position and has 2 or 3 double bonds and 17 carbon atoms; are expressed as sn-1-18:2$_{cis}$-PE and sn-1-18:3$_{cis}$-PE, respectively. The carboxylate carbon atom is the 18th carbon atom.

We have found that 1-oleoyl lysophosphatidylethanolamine (LOPE, or sn-1-18:1$_{cis}$-PE) exhibits a lamellar phase at physiological pH rather than the micellar arrangement of other lysophospholipids. LOPE, however, exhibits a micellar state at higher pH (i.e., at about 8.5 or higher) which promotes micellar solubilization of hydrophobic substances under such conditions. This polymorphic phase behavior from micellar to bilayer states is substantiated by: (a) ^{31}P-NMR spectra, which correspond to a lamellar configuration at pH 7 at temperatures of -20°C to 90°C, in contrast to lysophosphatidylcholine which is micellar (Fig. 1); (b) x-ray diffraction patterns of LOPE, in which the x-ray scatter forms equidistantly spaced rings, indicative of a lamellar organization; (c) freeze fracture micrographs which show the unilamellar nature of LUVETS produced at pH 7.0; and (d) ^{31}P-NMR spectra that show isotropic motional averaging at pH 9.0, indicative of a micellar structure (Fig. 2). This polymorphic phase behavior allows micellar solubilization of a hydrophobic material at a pH of 8.5, and bilayer formation at lower pH.

We have also found that lysophosphatidylethanolamines having 2 or 3 double bonds such as sn-1-18:2$_{cis}$-PE and sn-1-18:3$_{cis}$-PE, respectively, exhibit polymorphic phase behavior in response to temperature variation. Below 0°C, both lipids give rise to ^{31}P-NMR spectra indicative of overall lamellar organization indicated by a low field shoulder followed by a peak (Fig. 3). Both sn-1-18:2$_{cis}$-PE and sn-1-18:3$_{cis}$-PE show a hexagonal structure at 0-1°C. However, at 10-20°C and above, both lipids are in the (inverted) micelle or H_I state.

In the present invention, a lipid film and hydrophobic material are mixed in an aqueous medium resulting in solubilization of the hydrophobic material. This solubilization may be achieved by several methods. In the case where LOPE is the solubilizing lipid used, the hydrophobic material is combined with LOPE in an amount sufficient to solubilize it, and both dried to a film in a receptacle, from an organic solvent. Suitable organic solvents are those with a variety of polarities and dielectric properties, including chloroform, acetone, methylene chloride, diethyl and petroleum ethers, and mixtures of chloroform and methanol. All of the above-mentioned solvents will dissolve the phospholipids. The dry film is then hydrated with an aqueous medium at pH of between 8.2 and 14.0. Alternatively, a dry film of LOPE may be hydrated with an aqueous medium at pH of between 8.2 and 14.0, followed by addition of the hydrophobic material. Lastly, an aqueous medium at pH of between 8.2 and 14.0 containing a hydrophobic material may be used to hydrate a dry film of LOPE. The hydrophobic material may be a bioactive agent.

In the preferred embodiment, lipid and hydrophobic material are dried under vacuum from chloroform to a thin film. The dried film is then hydrated with an aqueous buffer such as borate, HEPES, or potassium glutarate (KGlu) at pH 8.5-14.0; most preferably pH 8.5. In general, in the 8.5-14.0 pH range, lysophosphatidylethanolamine assumes its micellar state. At pH of about 8.0 and lower, LOPE is in a lamellar state. The hydrophobic material is rehydrated with the lipid in an aqueous medium with agitation and/or vortical mixing. The concentration of hydrophobic material can preferably range from 5-25 mg/ml of buffer. The LOPE dispersion was held at 4°C for 2-3 hours to favor micellization. This dispersion is

optionally then subjected to 10 repeated extrusions at pressures of 4827 kPa (700 psi) using an extrusion apparatus; this method and "LUVET" apparatus described in a copending application, Serial No. 622,690, filed June 20, 1984, Pieter R. Cullis et.al., "Extrusion Technique for Producing Unilamellar Vesicles", relevant portions of which are incorporated herein by reference. The samples were held at 20-30°C, preferably 25°C for 16-18 hours to confirm complete solubilization, evidenced by lack of precipitation. The solubilized product can be used as an injectable product administered, for example, intravenously, intramuscularly, or subcutaneously, in a subject including mammals such as humans. The product is best used in the form of a sterile aqueous solution which may contain other solutes, for example, enough salts or glucose to make the solution isotonic.

In cases where sn-1-18:2_{cis}-PE or sn-1-18:3_{cis}-PE's are the solubilizing lipids used, the hydrophobic material may be combined with the lipid in an amount sufficient to solubilize it, and both dried to a film in a receptacle, from organic solvent. The dry film may then be hydrated with an aqueous medium at pH between 6.0 and 8.0 and held at a temperature from -90°C to 0°C, preferably -20°C, which favors the lamellar phase of the lipid. Alternatively, a dry lipid film may be hydrated with an aqueous medium at pH of between 6.0 and 8.0 followed by addition of or combined with the hydrophobic material. The solubilized product may be stored at this reduced temperature, in lamellar phase lipid for purposes of enhancing shelf life.

MATERIALS AND METHODS

Dioleoyl phosphatidylethanolamine (DOPE) was prepared from dioleoyl PC according to established procedures, Confurius, P. et al., Biophys. Biochem. Acta., 488, 36-42 (1977). Lysophosphatidylethanolamine was prepared according to the following protocol: 500 mg DOPE was dissolved in 50 ml anhydrous diethyl ether to which was added 10 ml 0.5M Tris/HCl buffer (pH 7.4), 10 ml of 2.5 mM $CaCl_2$ and 100 mg of Crotalus adamanteus venom (Sigma Chemical Co., St. Louis, MO). The reaction vessel was flushed with nitrogen, sealed, covered with aluminum foil, and stirred vigorously at room temperature. The mixture was rotoevaporated under reduced pressure to remove the diethyl ether and the aqueous phase extracted with chloroform methanol 2:1 v/v followed by chloroform. The preparation was purified by liquid chromatography using a Waters Prep 500 LC unit. Purity was further verified by H-NMR.

The present invention is exemplified by the following Examples, but the scope of the invention is not limited thereto.

EXAMPLE 1

Fifty µmol of LOPE combined with 5 mg of 21-acetoxypregnenolone (Sigma Chemical Co., St. Louis, MO) was rotoevaporated to a dried film from chloroform onto the inner sides of a test tube. One ml of borate buffer (100 mM $NaHCO_3$,50 mM borate), pH 8.5, was added to the tube and the lipid dispersed by vortexing. After dispersion by vortexing, the preparations were left to stand at 4°C for 2-3 hours. The dispersions were then transferred into the pressure chamber of the LUVET apparatus, equipped with two stacked 100 nm polycarbonate filters. Positive pressure was applied to the chamber by way of a standard nitrogen cylinder at 3500 kPa (500 psi). The pressure was adjusted within the 1400-6300 kPa (200-900 psi) range to allow complete extrusion of the sample without the membrane filter clogging. Application of pressure results in the extrusion of the sample through the filters. Each sample was extruded a total of ten times.

The above procedures were repeated using 10, 15, 20, and 25 mg of 21-acetoxypregnenolone.

The samples were left at 25°C for 16-18 hours, after which time all samples appeared clear; viscosity increasing with increasing 21-acetoxypregnenolone content.

EXAMPLE 2

The procedures and materials of Example 1 were employed using LOPE and DOPC in a 1:1 molar ratio to solubilize 10 mg of 21-acetoxypregnenolone. Following the LUVET treatment, the solution was allowed to stand for 16-18 hours at 25°C, and a further LUVET filtration employing a 50 nm filter system at a pressure of 1400 kPa (200) psi was performed. Following this treatment, no particulate material was observed on the filter, and the preparation appeared translucent. Attempts to solubilize 20 mg of steroid in 1.0 ml of buffer were unsuccessful.

EXAMPLE 3

Hemolytic properties of 1-oleoyl lysophosphatidylethanolamine (LOPE) were tested at both pH 7.0 and 8.5, and compared to those of 1-oleoyl lysophosphatidylcholine (LOPC) and dipalmitoyl-phosphatidylethanolamine (DPPE). LOPE, LOPC, and DPPE stock solutions were made at 12-13 mg/ml in 50 mM borate buffer at pH 7.0 and 8.5. Following the additions of 0.1 ml volumes of stock solution of one of these compounds to 1.0 ml aliquots of heparinized whole blood, hemolytic activities were assayed by spectrophotometric measurement of the centrifuged supernatants at 550 nm.

FIG. 4 shows the reduced hemolytic activity of LOPE at both pH 7.0 and 8.5 as compared to LOPC, when added to whole blood. LOPC produced the greatest amount of red blood cell lysis, liberating the most hemoglobin. High concentrations of LOPE produced lysis as compared to DPPE, which caused minimal lysis (FIG. 5). Control experiments with borate buffer alone at both pH 7.0 and 8.5 produced no lysis.

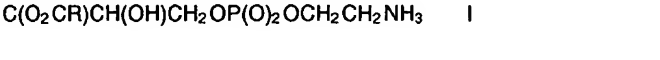
EXAMPLE 4

Fifty μmol of sn-1-18:2_{cis}-PE combined with 5 mg of 21-acetoxypregnenolone is rotoevaporated to a dry film from chloroform onto the inner sides of a test tube. One ml of borate buffer, pH 7.0, is added to the tube and the lipid dispersed by vortical mixing. After dispersion, the suspension is cooled to 20°C, and transferred into the pressure chamber of the LUVET and extruded using the procedures of Example 1.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A composition comprising an aqueous micellar solution at between pH 8.2 to 14.0 of a hydrophobic material and a lysophospholipid of the formula :



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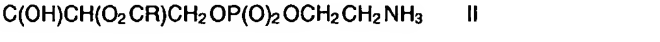
or

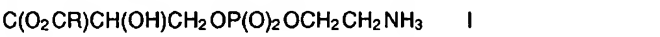


II

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 1 double bond.

2. The composition according to claims 1 and 2 wherein the aqueous solution is at pH of 8.5.

3. A composition comprising an aqueous micellar solution at between about pH 6.0 to 8.0 of a hydrophobic material and a lysophospholipid of the formula :



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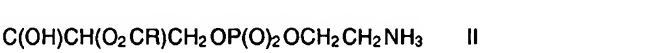


II

wherein R is a hydrocarbon chain having between about 11 and 21 carbon atoms and 2 to 6 double bonds, and wherein the temperature of the composition is between - 20°C and 0°C.

4. The composition according to claim 3 wherein the aqueous solution is at a pH of 7.0.

5. The composition of claims 1 to 4 wherein the lysophosphatidylethanolamine has formula I.

6. The composition of claims 1 to 5 wherein R has between 13 to 19 carbon atoms.

7. The composition of claims 1 to 6 wherein R has between 15 and 17 carbon atoms.

8. The composition of claim wherein R has 3 double bonds.

9. The composition of claims 3 to 7 wherein R has between 15 and 17 carbon atoms, and 2 or 3 double bonds.

10. The composition of claims 1 to 9 where RCO_2 is 1-oleoyl.

11. The composition of claims 1 to 10 wherein the hydrophobic material is a bioactive agent.

12. The composition of claims 1 to 11 wherein the aqueous solution comprises aqueous buffer.

13. The composition according to claims 1 to 12 additionally comprising an unsaturated phospholipid.

14. The composition of claim 13 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between 12 and 22 carbon atoms and 1 to 6 double bonds.

15. The composition of claims 13 and 14 wherein a second fatty acid chain of the unsaturated phospholipid comprises between 12 and 22 carbon atoms and 0 to 6 double bonds.

16. The composition of claims 13 to 15 wherein the fatty acid chain of the unsaturated phospholipid has between 16 to 20 carbon atoms and 1 to 3 double bonds.

17. The composition of claims 13 to 16 wherein the unsaturated phospholipid is selected from egg phosphatidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

18. A composition according to claims 1 to 17 which can be parenterally administered.

19. The composition according to claims 1 to 18 which can be parenterally administered to a mammal.

20. A method of preparation of a composition according to claims 1 to 2 and 5 to 19, which comprises solubilizing a hydrophobic material with the steps of :

(a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of a lysophosphatidylethanolamine of the formula :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

and a hydrophobic material are dissolved to obtain a film ; and

(b) hydrating the film with an aqueous medium at pH of between 8.2 and 14.0 ; and

(c) admixing the aqueous medium and the hydrated film comprising the hydrophobic material and the lysophosphatidylethanolamine,

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 1 double bond.

21. The method according to claim 20 wherein the aqueous medium is at pH of 8.5.

22. A method of preparation of a composition according to claims 3 to 19 which comprises solubilizing a hydrophobic material with the steps of :

(a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of lysophosphatidylethanolamine of the formula :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

or

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

is dissolved to obtain a film ;

(b) hydrating the film with an aqueous medium at pH of between 6.0 and 8.0 ;

(c) admixing the hydrophobic material with the hydrated film of step (b); and

(d) cooling the dispersed lipid in aqueous medium to a temperature below 0 ° C ;

wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 2 to 6 double bonds.

23. The method according to claim 22 wherein the aqueous medium is at pH of 7.0.

24. The method of claims 20 to 23 wherein the step (b) is followed by the step of adding the hydrophobic material.

25. The method of claims 20 to 24 wherein the hydrophobic material is combined with the aqueous medium prior to hydrating the film in step (b).

26. The method according to claims 20 to 25 comprising the additional step of filtering the product of the last step.

Claims for the following Contracting State : AT

1. A method of preparation of a composition which comprises solubilizing a hydrophobic material with the steps of :
   (a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of a lysophosphatidylethanolamine of the formula:

   $H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

   or

   $H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

   and a hydrophobic material is dissolved to obtain a film ; and
   (b) hydrating the film with an aqueous medium at pH of between 8.2 and 14.0 ; and
   (c) admixing the aqueous medium and the hydrated film comprising the hydrophobic material and the lysophosphatidylethanolamine,
   wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 1 double bond.

2. The method according to claim 1 wherein the aqueous medium is at pH of 8.5.

3. A method of preparation of a composition which comprises solubilizing a hydrophobic material with the steps of :
   (a) removing an organic solvent in which a composition comprising a hydrophobic-material-solubilizing effective amount of lysophosphatidylethanolamine of the formula :

   $H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

   or

   $H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

   is dissolved to obtain a film ;
   (b) hydrating the film with an aqueous medium at pH of between 6.0 and 8.0 ;
   (c) admixing the hydrophobic material with the hydrated film of step (b) ; and
   (d) cooling the dispersed lipid in aqueous medium to a temperature below 0 ° C ;
   wherein R is a hydrocarbon chain having between 11 and 21 carbon atoms and 2 to 6 double bonds.

4. The method according to claim 3 wherein the aqueous medium is at pH of 7.0.

5. The method of claims 1 to 4 wherein the step (b) is followed by the step of adding the hydrophobic material.

6. The method of claims 1 to 5 wherein the hydrophobic material is combined with the aqueous medium prior to hydrating the film in step (b).

7. The method according to claims 1 to 6 comprising the additional step of filtering the product of the last step.

8. The method of claims 1 to 7 wherein the lysophosphatidylethanolamine as formula I.

9. The method of claims 1 to 8 wherein R has between 13 to 19 carbon atoms.

10. The method of claims 1 to 9 wherein R has between 15 and 17 carbon atoms.

11. The method of claims 3 to 10 wherein R has 3 double bonds.

12. The method of claims 3 to 10 wherein R has between 15 and 17 carbon atoms, and 2 or 3 double bonds.

13. The method of claims 1 to 12 where RCO_2 is 1-oleoyl.

14. The method of claims 1 to 13 wherein the hydrophobic material is a bioactive agent.

15. The method of claims 1 to 14 wherein the aqueous solution comprises aqueous buffer.

16. The method according to claims 1 to 15 additionally comprising an unsaturated phospholipid.

17. The method of claim 16 wherein the unsaturated phospholipid comprises at least one unsaturated fatty acid chain of between 12 and 22 carbon atoms and 1 to 6 double bonds.

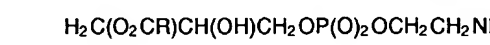
18. The method of claims 16 and 17 wherein a second fatty acid chain of the unsaturated phospholipid comprises between 12 and 22 carbon atoms and 0 to 6 double bonds.

19. The method of claims 16 to 18 wherein the fatty acid chain of the unsaturated phospholipid has between 16 to 20 carbon atoms and 1 to 3 double bonds.

20. The method of claims 16 to 19 wherein the unsaturated phospholipid id selected from egg phosphatidylcholine, soy phosphatidylcholine, and dioleoylphosphatidylcholine.

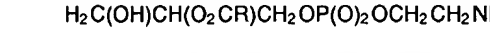
Revendications
Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Composition comprenant une solution micellaire aqueuse à pH compris entre 8,2 et 14,0, d'une matière hydrophobe et d'un lysophospholipide de formule :



I

ou

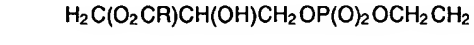


II

dans laquelle R est une chaîne hydrocarbonée ayant entre 11 et 21 atomes de carbone et une double liaison.

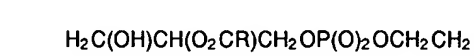
2. Composition selon les revendications 1 et 2, dans laquelle la solution aqueuse est à un pH de 8,5.

3. Composition comprenant une solution micellaire aqueuse à un pH compris entre 6,0 et 8,0 d'une matière hydrophobe et d'un lysophospholipide de formule :



I

ou



II

dans laquelle R est une chaîne hydrocarbonée ayant entre environ 11 et 21 atomes de carbone de 2 à

6 doubles liaisons, et où la température de la composition est comprise entre -20 °C et 0 °C.

4. Composition selon la revendication 3, dans laquelle la solution aqueuse est à un pH de 7,0.

5. Composition des revendications 1 à 4, dans laquelle la lysophosphatidyléthanolamine a la formule I.

6. Composition des revendications 1 à 5, dans laquelle R a de 13 à 19 atomes de carbone.

7. Composition des revendications 1 à 6, dans laquelle R a de 15 à 17 atomes de carbone.

8. Composition de la revendication 3, dans laquelle R a trois doubles liaisons.

9. Composition des revendications 3 à 7, dans laquelle R a entre 15 et 17 atomes de carbone, et 2 ou 3 doubles liaisons.

10. Composition des revendications 1 à 9, dans laquelle RCO_2 est le 1-oléoyle.

11. Composition des revendications 1 à 10, dans laquelle la matière hydrophobe est un agent bioactif.

12. Composition des revendications 1 à 11, dans laquelle la solution aqueuse comprend un tampon aqueux.

13. Composition selon les revendications 1 à 12, comprenant en outre un phospholipide insaturé.

14. Composition de la revendication 13, dans laquelle le phospholipide insaturé comprend au moins une chaîne acide gras insaturée comprenant entre 12 et 22 atomes de carbone et de 1 à 6 doubles liaisons.

15. Composition des revendications 13 et 14, dans laquelle une seconde chaîne d'acide gras du phospholipide insaturé comprend entre 12 et 22 atomes de carbone et de 0 à 6 doubles liaisons.

16. Composition des revendications 13 à 15, dans laquelle la chaîne acide gras du phospholipide insaturé a de 16 à 20 atomes de carbone et de 1 à 3 doubles liaisons.

17. Composition des revendications 13 à 16, dans laquelle le phospholipide insaturé est choisi parmi la phosphatidylcholine d'oeuf, la phosphatidylcholine de soja et la dioléoylphosphatidylcholine.

18. Compositions selon les revendications 1 à 17, qui peuvent être administrées par voie parentérale.

19. Compositions selon les revendications 1 à 18 qui peuvent être administrées par voie parentérale à un mammifère.

20. Procédé de préparation d'une composition selon les revendications 1 à 2, et 5 à 19, dans lequel on solubilise une matière hydrophobe avec les étapes de :
(a) élimination d'un solvant organique dans lequel une composition comprenant une quantité efficace pour solubiliser une matière hydrophobe d'une lysophosphatidyléthanolamine de formule :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

ou

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

et d'une matière hydrophobe est dissoute pour obtenir une pellicule ; et
(b) hydratation de la pellicule avec un milieu aqueux à un pH compris entre 8,2 et 14,0 ; et
(c) mélange du milieu aqueux et de la pellicule hydratée comprenant la matière hydrophobe et la lysophosphatidyléthanolamine,
où R est une chaîne hydrocarbonée ayant entre 11 et 21 atomes de carbone et une double liaison.

21. Procédé selon la revendication 20 dans lequel le milieu aqueux est à un pH de 8,5.

22. Procédé de préparation d'une composition selon les revendications 3 à 19 dans lequel on solubilise une matière hydrophobe avec les étapes de :
(a) élimination d'un solvant organique dans lequel une composition comprenant une quantité efficace pour solubiliser une matière hydrophobe de lysophosphatidyléthanolamine de formule :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

ou

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

est dissoute pour obtenir une pellicule ;
(b) hydratation de la pellicule avec un milieu aqueux à un pH compris entre 6,0 et 8,0 ;
(c) mélange de la matière hydrophobe avec la pellicule hydratée de l'étape (b) ; et
(d) refroidissement du lipide dispersé dans un milieu aqueux à une température inférieure à 0 ° C ;
où R est une chaîne hydrocarbonée ayant entre 11 et 21 atomes de carbone et de 2 à 6 doubles liaisons.

23. Procédé selon la revendication 22, dans lequel le milieu aqueux est à un pH de 7,0.

24. Procédé des revendications 20 à 23, dans lequel l'étape (b) est suivie par l'étape d'addition de la matière hydrophobe.

25. Procédé des revendications 20 à 24, dans lequel la matière hydrophobe est combinée avec le milieu aqueux avant l'hydratation de la pellicule dans l'étape (b).

26. Procédé selon les revendications 20 à 25 comprenant l'étape additionnelle de filtration du produit de la dernière étape.

Revendications pour l'Etat contractant suivant : AT

1. Procédé de préparation d'une composition dans lequel on solubilise une matière hydrophobe avec les étapes de :
(a) élimination d'un solvant organique dans lequel une composition comprenant une quantité efficace pour solubiliser une matière hydrophobe d'une lysophosphatidyléthanolamine de formule :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

ou

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

et d'une matière hydrophobe est dissoute pour obtenir une pellicule ; et
(b) hydratation de la pellicule avec un milieu aqueux à un pH compris entre 8,2 et 14,0 ; et
(c) mélange du milieu aqueux et de la pellicule hydratée comprenant la matière hydrophobe et la lysophosphatidyléthanolamine,
où R est une chaîne hydrocarbonée ayant entre 11 et 21 atomes de carbone et une double liaison.

2. Procédé selon la revendication 1, dans lequel le milieu aqueux est à un pH de 8,5.

3. Procédé de préparation d'une composition, dans lequel on solubilise une matière hydrophobe avec les étapes de :
(a) élimination d'un solvant organique dans lequel une composition comprenant une quantité efficace pour solubiliser une matière hydrophobe de lysophosphatidyléthanolamine de formule :

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

ou

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

est dissoute pour obtenir une pellicule ;
(b) hydratation de la pellicule avec un milieu aqueux à un pH compris entre 6,0 et 8,0 ;
(c) mélange de la matière hydrophobe avec la pellicule hydratée de l'étape (b) ; et
(d) refroidissement du lipide dispersé dans un milieu aqueux à une température inférieure à 0 °C ;
où R est une chaîne hydrocarbonée ayant entre 11 et 21 atomes de carbone et de 2 à 6 doubles liaisons.

4. Procédé selon la revendication 3, dans lequel le milieu aqueux est à un pH de 7,0.

5. Procédé des revendications 1 à 4, dans lequel l'étape (b) est suivie par l'étape d'addition de la matière hydrophobe.

6. Procédé des revendications 1 à 5, dans lequel la matière hydrophobe est combinée avec le milieu aqueux avant l'hydratation de la pellicule dans l'étape (b).

7. Procédé selon les revendications 1 à 6, comprenant l'étape additionnelle de filtration du produit de la dernière étape.

8. Procédé des revendications 1 à 7, dans lequel la lysophosphatidyléthanolamine est de formule I.

9. Procédé des revendications 1 à 8, dans lequel R a entre 13 et 19 atomes de carbone.

10. Procédé des revendications 1 à 9, dans lequel R a entre 15 et 17 atomes de carbone.

11. Procédé des revendications 3 à 10, dans lequel R a trois doubles liaisons.

12. Procédé des revendications 3 à 10 dans lequel R a entre 15 et 17 atomes de carbone et 2 ou 3 doubles liaisons.

13. Procédé des revendications 1 à 12, dans lequel RCO_2 est le 1-oléoyle.

14. Procédé des revendications 1 à 13, dans lequel la matière hydrophobe est un agent bioactif.

15. Procédé des revendications 1 à 14, dans lequel la solution aqueuse comprend un tampon aqueux.

16. Procédé selon les revendications 1 à 15, comprenant en outre un phospholipide insaturé.

17. Procédé de la revendication 16, dans lequel le phospholipide insaturé comprend au moins une chaîne acide gras insaturée comprenant entre 12 et 22 atomes de carbone et de 1 à 6 doubles liaisons.

18. Procédé des revendications 16 et 17, dans lequel une seconde chaîne acide gras du phospholipide insaturé comprend entre 12 et 22 atomes de carbone et 0 à 6 doubles liaisons.

19. Procédé des revendications 16 à 18, dans lequel la chaîne acide gras du phospholipide insaturé a entre 16 et 20 atomes de carbone et 1 à 3 doubles liaisons.

20. Procédé des revendications 16 à 19, dans lequel le phospholipide non saturé est choisi parmi la phosphatidylcholine d'oeuf, la phosphatidylcholine de soja et la dioléoylphosphatidylcholine.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Zusammensetzung, umfassend eine wäßrige micellare Lösung mit einem pH-Wert von 8,2 bis 14,0, eines hydrophoben Materials und eines Lysophospolipids der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

worin R eine Kohlenwasserstoffkette mit 11 bis 21 Kohlenstoffatomen und 1 Doppelbindung ist.

2. Zusammensetzung nach den Ansprüchen 1 und 2, worin die wäßrige Lösung den pH-Wert 8,5 hat.

3. Zusammensetzung, umfassend eine wäßrige micellare Lösung mit einem pH-Wert von etwa 6,0 bis 8,0, eines hydrophoben Materials und eines Lysophospholipids der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

worin R eine Kohlenwasserstoffkette mit etwa 11 bis 21 Kohlenstoffatomen und 2 bis 6 Doppelbindungen ist und worin die Temperatur der Zusammensetzung bei -20 °C bis 0 °C liegt.

4. Zusammensetzung nach Anspruch 3, worin die wäßrige Lösung einen pH-Wert von 7,0 hat.

5. Zusammensetzung nach den Ansprüchen 1 bis 4, worin das Lysophosphatidylethanolamin die Formel I hat.

6. Zusammensetzung nach den Ansprüchen 1 bis 5, worin R 13 bis 19 Kohlenstoffatome hat.

7. Zusammensetzung nach den Ansprüchen 1 bis 6, worin R 15 bis 17 Kohlenstoffatome hat.

8. Zusammensetzung nach Anspruch 3, worin R 3 Doppelbindungen hat.

9. Zusammensetzung nach den Ansprüchen 3 bis 7, worin R 15 bis 17 Kohlenstoffatome und 2 oder 3 Doppelbindungen hat.

10. Zusammensetzung nach den Ansprüchen 1 bis 9, worin RCO_2 1-Oleoyl ist.

11. Zusammensetzung nach den Ansprüchen 1 bis 10, worin das hydrophobe Material ein bioaktives Mittel ist.

12. Zusammensetzung nach den Ansprüchen 1 bis 11, worin die wäßrige Lösung wäßrigen Puffer umfaßt.

13. Zusammensetzung nach den Ansprüchen 1 bis 12, die zusätzlich ein ungesättigtes Phospholipid umfaßt.

14. Zusammensetzung nach Anspruch 13, worin das ungesättigte Phospholipid mindestens eine ungesättigte Fettsäurekette mit 12 bis 22 Kohlenstoffatomen und 1 bis 6 Doppelbindungen umfaßt.

15. Zusammensetzung nach den Ansprüchen 13 und 14, worin eine zweite Fettsäurekette des ungesättigten Phospholipids 12 bis 22 Kohlenstoffatome und 0 bis 6 Doppelbindungen umfaßt.

16. Zusammensetzung nach den Ansprüchen 13 bis 15, worin die Fettsäurekette des ungesättigten Phospholipids 16 bis 20 Kohlenstoffatome und 1 bis 3 Doppelbindungen umfaßt.

17. Zusammensetzung nach den Ansprüchen 13 bis 16, worin das ungesättigte Phosholipid ausgewählt ist aus Ei-Phosphatidylcholin, Soja-Phosphatidylcholin und Dioleoylphosphatidylcholin.

18. Zusammensetzung nach den Ansprüchen 1 bis 17, die parenteral verabreicht werden kann.

19. Zusammensetzung nach den Ansprüchen 1 bis 18, die parenteral an einen Säuger verabreicht werden kann.

20. Verfahren zur Herstellung einer Zusammensetzung nach den Ansprüchen 1 bis 2 und 5 bis 19, das das Solubilisieren eines hydrophoben Materials mit folgenden Stufen umfaßt:
a) Entfernen eines organischen Lösungsmittels, in dem eine Zusammensetzung, umfassend eine zum Solubilisieren von hydrophobem Material wirksame Menge eines Lysophoshatidylethanolamins der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

und ein hydrophobes Material gelöst sind, unter Bildung eines Films; und
b) Hydratisieren des Films mit einem wäßrigen Medium beim pH-Wert von 8,2 bis 14,0; und
c) Vermischen des wäßrigen Mediums und des hydratisierten Films, der das hydrophobe Material und das Lysophosphatidylethanolamin enthält,
worin R eine Kohlenwasserstoffkette mit 11 bis 21 Kohlenstoffatomen und 1 Doppelbindung ist.

21. Verfahren nach Anspruch 20, worin das wäßrige Medium den pH-Wert 8,5 hat.

22. Verfahren zur Herstellung einer Zusammensetzung nach den Ansprüchen 3 bis 19, das das Solubilisieren eines hydrophoben Materials mit folgenden Stufen umfaßt:
a) Entfernen eines organischen Lösungsmittels, in dem eine Zusammensetzung, umfassend eine zum Solubilisieren von hydrophobem Material wirksame Menge an Lysophosphatidylethanolamin der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

gelöst ist, unter Erzielung eines Films;
b) Hydratisieren des Films mit einem wäßrigen Medium beim pH-Wert von 6,0 bis 8,0;
c) Vermischen des hydrophoben Materials mit dem hydratisierten Film der Stufe (b); und
d) Kühlen des dispergierten Lipids in wäßrigem Medium auf eine Temperatur unter 0 °C;
worin R eine Kohlenwasserstoffkette mit 11 bis 21 Kohlenstoffatomen und 2 bis 6 Doppelbindungen ist.

23. Verfahren nach Anspruch 22, worin das wäßrige Medium den pH-Wert 7,0 hat.

24. Verfahren nach den Ansprüchen 20 bis 23, worin der Stufe (b) die Stufe des Zusatzes des hydrophoben Materials folgt.

25. Verfahren nach den Ansprüchen 20 bis 24, worin das hydrophobe Material mit dem wäßrigen Medium vor dem Hydratisieren des Films in der Stufe (b) kombiniert wird.

26. Verfahren nach den Ansprüchen 20 bis 25, umfassend die zusätzliche Stufe des Filtrierens des Produkts der letzten Stufe.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung einer Zusammensetzung, das das Solubilisieren eines hydrophoben Materials mit folgenden Stufen umfaßt:

a) Entfernen eines organischen Lösungsmittels, in dem eine Zusammensetzung, umfassend eine zum Solubilisieren von hydrophobem Material wirksame Menge eines Lysophoshatidylethanolamins der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

und ein hydrophobes Material gelöst sind, unter Bildung eines Films; und
b) Hydratisieren des Films mit einem wäßrigen Medium beim pH-Wert von 8,2 bis 14,0; und
c) Vermischen des wäßrigen Mediums und des hydratisierten Films, der das hydrophobe Material und das Lysophosphatidylethanolamin enthält,
worin R eine Kohlenwasserstoffkette mit 11 bis 21 Kohlenstoffatomen und 1 Doppelbindung ist.

2. Verfahren nach Anspruch 1, worin das wäßrige Medium den pH-Wert 8,5 hat.

3. Verfahren zur Herstellung einer Zusammensetzung, das das Solubilisieren eines hydrophoben Materials mit folgenden Stufen umfaßt:
a) Entfernen eines organischen Lösungsmittels, in dem eine Zusammensetzung, umfassend eine zum Solubilisieren von hydrophobem Material wirksame Menge an Lysophosphatidylethanolamin der Formel

$H_2C(O_2CR)CH(OH)CH_2OP(O)_2OCH_2CH_2NH_3$ I

oder

$H_2C(OH)CH(O_2CR)CH_2OP(O)_2OCH_2CH_2NH_3$ II

gelöst ist, unter Erzielung eines Films;
b) Hydratisieren des Films mit einem wäßrigen Medium beim pH-Wert vor 6,0 bis 8,0;
c) Vermischen des hydrophoben Materials mit dem hydratisierten Film der Stufe (b); und
d) Kühlen des dispergierten Lipids in wäßrigem Medium auf eine Temperatur unter 0 °C;
worin R eine Kohlenwasserstoffkette mit 11 bis 21 Kohlenstoffatomen und 2 bis 6 Doppelbindungen ist.

4. Verfahren nach Anspruch 3, worin das wäßrige Medium den pH-Wert 7,0 hat.

5. Verfahren nach den Ansprüchen 1 bis 4, worin der Stufe (b) die Stufe des Zusatzes des hydrophoben Materials folgt.

6. Verfahren nach den Ansprüchen 1 bis 5, worin das hydrophobe Material mit dem wäßrigen Medium vor dem Hydratisieren des Films in der Stufe (b) kombiniert wird.

7. Verfahren nach den Ansprüchen 1 bis 6, umfassend die zusätzliche Stufe des Filtrierens des Produkts der letzten Stufe.

8. Verfahren nach den Ansprüchen 1 bis 7, worin das Lysophosphatidylethanolamin die Formel I hat.

9. Verfahren nach den Ansprüchen 1 bis 8, worin R 13 bis 19 Kohlenstoffatome hat.

10. Verfahren nach den Ansprüchen 1 bis 9, worin R 15 bis 17 Kohlenstoffatome hat.

11. Verfahren nach den Ansprüchen 3 bis 10, worin R 3 Doppelbindungen hat.

12. Verfahren nach den Ansprüchen 3 bis 10, worin R 15 bis 17 Kohlenstoffatome und 2 oder 3 Doppelbindungen hat.

13. Verfahren nach den Ansprüchen 1 bis 12, worin RCO_2 1-Oleoyl ist.

14. Verfahren nach den Ansprüchen 1 bis 13, worin das hydrophobe Material ein bioaktives Mittel ist.

15. Verfahren nach den Ansprüchen 1 bis 14, worin die wäßrige Lösung wäßrigen Puffer umfaßt.

16. Verfahren nach den Ansprüchen 1 bis 15, die zusätzlich ein ungesättigtes Phospholipid umfaßt.

17. Verfahren nach Anspruch 16, worin das ungesättigte Phospholipid mindestens eine ungesättigte Fettsäurekette mit 12 bis 22 Kohlenstoffatomen und 1 bis 6 Doppelbindungen umfaßt.

18. Verfahren nach den Ansprüchen 16 und 17, worin eine zweite Fettsäurekette des ungesättigten Phospholipids 12 bis 22 Kohlenstoffatome und 0 bis 6 Doppelbindungen umfaßt.

19. Verfahren nach den Ansprüchen 16 bis 18, worin die Fettsäurekette des ungesättigten Phospholipids 16 bis 20 Kohlenstoffatome und 1 bis 3 Doppelbindungen umfaßt.

20. Verfahren nach den Ansprüchen 16 bis 19, worin das ungesättigte Phospholipid ausgewählt wird aus Ei-Phosphatidylcholin, Soja-Phosphatidylcholin und Dioleoylphosphatidylcholin.

FIG. 1

^{31}P-NMR SPECTRA OF LOPE AT pH 7

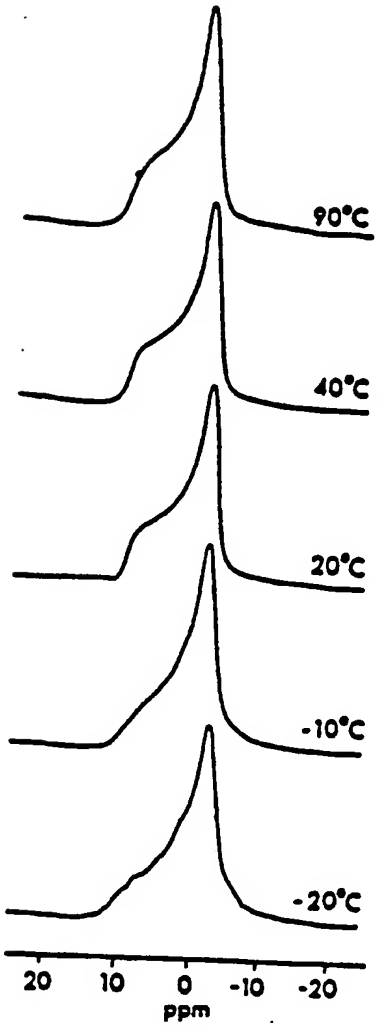


FIG. 2

^{31}P-NMR SPECTRA OF pH DEPENDENT POLYMORPHIC PHASE BEHAVIOR OF LOPE

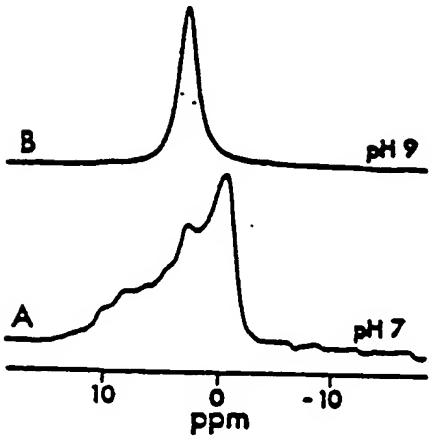
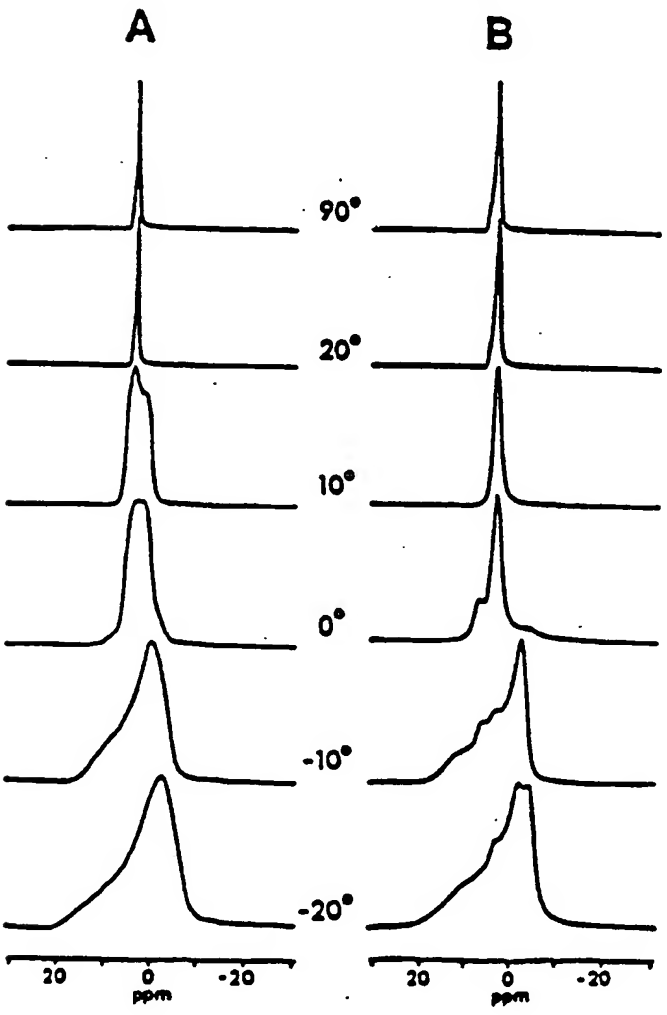


FIG. 3

^{31}P-NMR SPECTRA OF TEMPERATURE-DEPENDENT POLYMORPHIC PHASE BEHAVIOR OF sn-1-18:2$_{cis}$-PE AND sn-1-18:3$_{cis}$-PE



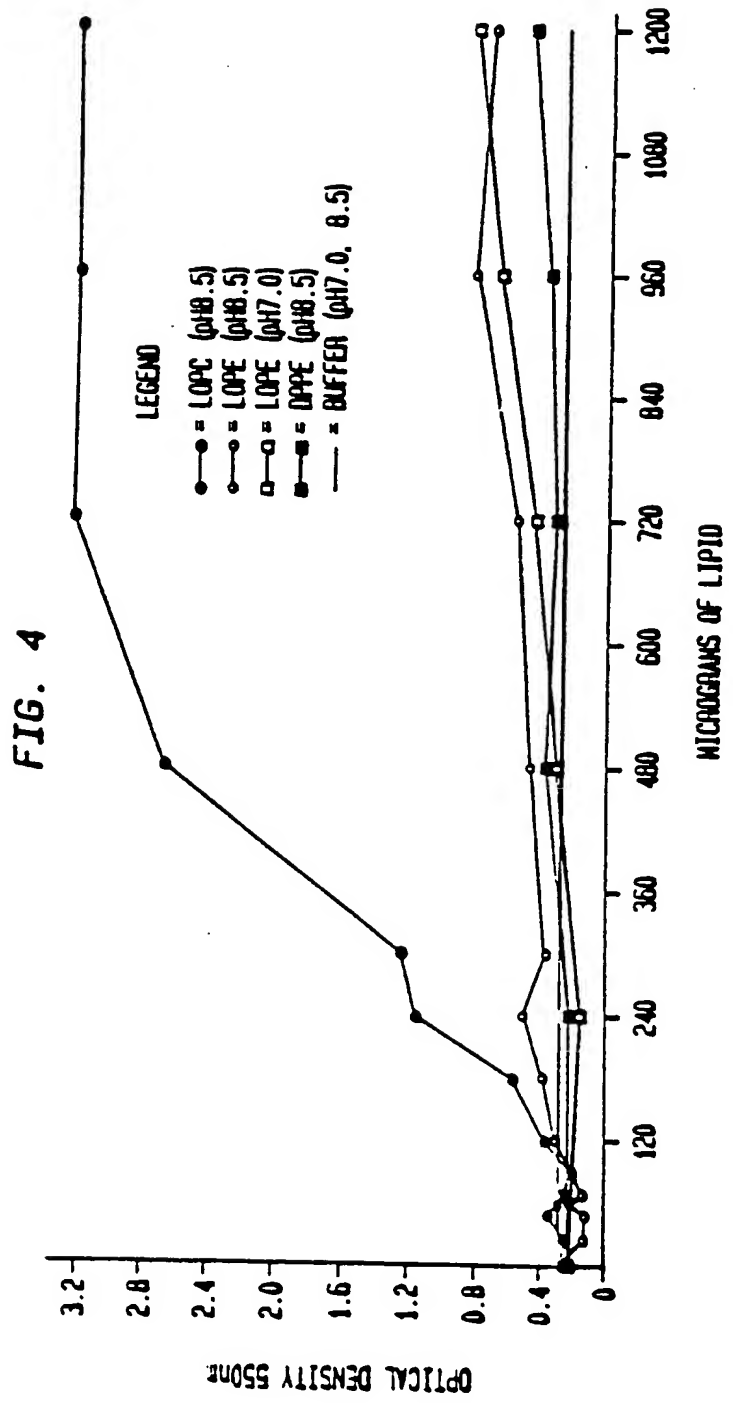
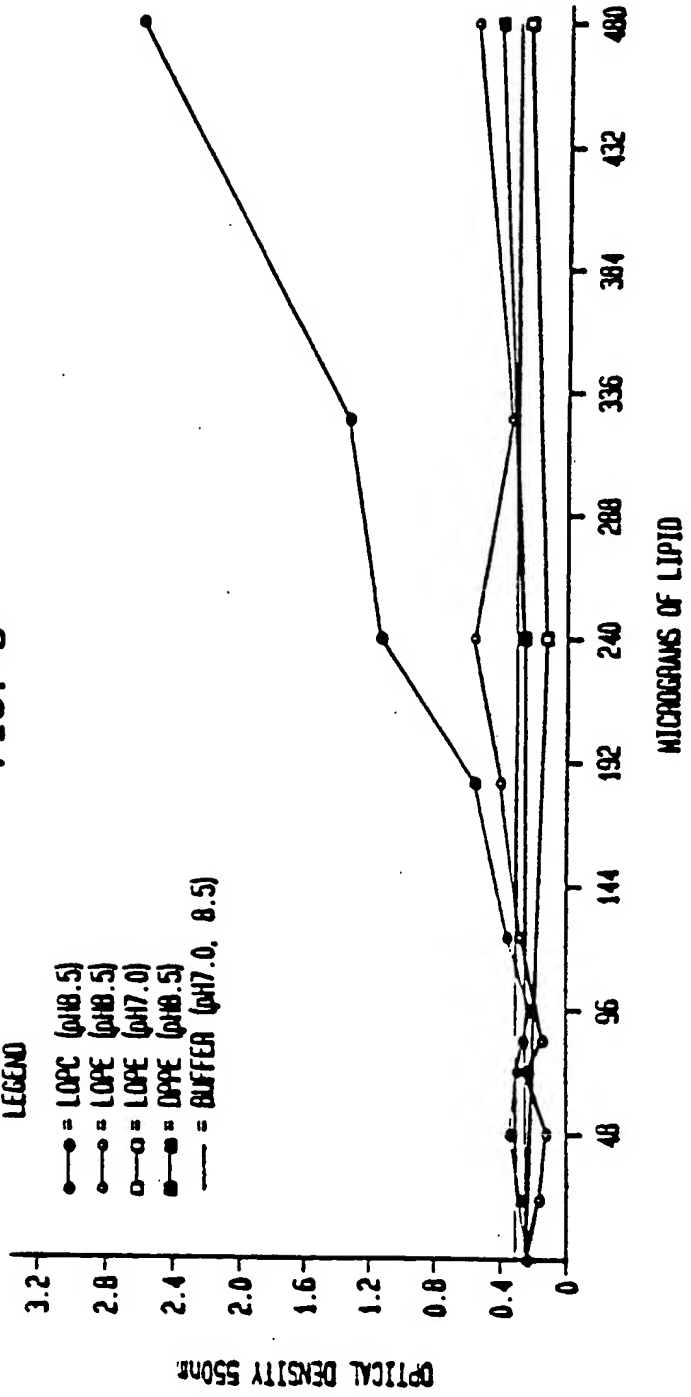

LEGEND
= LOPC (pH8.5)
= LOPE (pH8.5)
= LOPE (pH7.0)
= DAPE (pH8.5)
= BUFFER (pH7.0, 8.5)
OPTICAL DENSITY 550nm
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
120
240
360
480
600
720
840
960
1080
1200
MICROGRAMS OF LIPID

FIG. 4

FIG. 5


LEGEND
● = LOPC (pH8.5)
○ = LOPE (pH8.5)
□ = LOPE (pH7.0)
■ = DPPE (pH8.5)
— = BUFFER (pH7.0, 8.5)
OPTICAL DENSITY 550nm
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
48
96
144
192
240
288
336
384
432
480
MICROGRAMS OF LIPID